Neurochemistry International 88 (2015) 6–9

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Opposing roles of glutaminase isoforms in determining glioblastoma cell phenotype

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ARTICLE INFO

Article history:
Received 9 September 2014
Received in revised form 28 October 2014
Accepted 4 November 2014
Available online 18 December 2014

Keywords:
GLS
GLS2
Glioblastoma phenotype

ABSTRACT

Glutamine (Gln) and glutamate (Glu) play pivotal roles in the malignant phenotype of brain tumors *via* multiple mechanisms. Glutaminase (GA, EC 3.5.1.2) metabolizes Gln to Glu and ammonia. Human GA isoforms are encoded by two genes: *GLS* gene codes for kidney-type isoforms, KGA and GAC, whereas *GLS2* codes for liver-type isoforms, GAB and LGA. The expression pattern of both genes in different neoplastic cell lines and tissues implicated that the kidney-type isoforms are associated with cell proliferation, while the liver-type isoforms dominate in, and contribute to the phenotype of quiescent cells. *GLS* gene has been demonstrated to be regulated by oncogene c-Myc, whereas *GLS2* gene was identified as a target gene of p53 tumor suppressor. In glioblastomas (GBM, WHO grade IV), the most aggressive brain tumors, high levels of *GLS* and only traces or lack of *GLS2* transcripts were found. Ectopic overexpression of *GLS2* in human glioblastoma T98G cells decreased their proliferation and migration and sensitized them to the alkylating agents often used in the chemotherapy of gliomas. *GLS* silencing reduced proliferation of glioblastoma T98G cells and strengthen the antiproliferative effect evoked by previous *GLS2* overexpression.

1. Brain tumors

Gliomas account for more than 70% of all neoplasms of central nervous system (Ohgaki and Kleihues, 2009). They arise from astrocytes, oligodendrocytes and ependymocytes. Standard of care for gliomas consist of surgical resection followed by radiotherapy and treatment with the alkylating agents, mainly temozolomide (TMZ) (Stupp et al., 2005). Despite the progress in understanding of biology of gliomagenesis made during the past decade, the prognosis for patients with diffuse gliomas (WHO grades II–IV) are poor, with median survival times of 5.6 years for low-grade astrocytoma (WHO II), to less than 1 year for glioblastoma (WHO IV) (Ohgaki and Kleihues, 2005).

2. Glutamine in gliomas

Glutamine (Gln) plays a crucial role in the metabolism of neoplastic cells and tissues, including gliomas. This amino acid participates in cell bioenergetics and is a source of nitrogen for the production of molecules essential for cell growth (Cheng et al., 2011). It also facilitates cell migration by modulation of glioma cell volume (Ernest and Sontheimer, 2007). Moreover, Gln is a substrate for synthesis of glutamate (Glu) whose contribution to malignant phenotype of glioblastoma has been well documented. Significant quantities of Glu released by these tumors into the extracellular space exert peritumoral excitotoxicity and thus promote tumor expansion (Sontheimer, 2008). Glu is an essential precursor for synthesis of antioxidant glutathione (GSH). GSH level is elevated in glioblastoma and is partially responsible for decreased sensitivity of these tumors to oxidative stress and exposure to chemotherapeutic compounds (Ali-Osman et al., 1990; Iida et al., 1997).

3. Mammalian glutaminases

Phosphate-activated glutaminase (GA, EC 3.5.1.2), the enzyme converting Gln into glutamate (Glu) and ammonia, has received considerable attention in tumor biology. In mammals, there are two genes coding for GA: the *Gls* gene encodes kidney-type (K-type) isozymes, whereas the Gls2 gene encodes liver-type (L-type) isozymes (Aledo et al., 2000). Two K-type transcripts have been identified: KGA transcript is expressed in all mammalian tissues except liver (Curthoys and Watford, 1995), and an alternatively spliced variant, GAC, is expressed in heart, pancreas, kidneys, lungs, and breast cancer cells (Elgadi et al., 1999). The L-type transcript named LGA was originally cloned from rat liver (Smith and Watford, 1990). Later studies identified the second L-type transcript, recently termed GAB (de la Rosa et al., 2009), expressed in liver, brain, pancreas, breast carcinoma cells and cells of the immune system (Gomez-Fabre et al., 2000; Pérez-Gómez et al., 2005; Turner and McGivan, 2003).

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http://dx.doi.org/10.1016/j.neuint.2014.11.004
0197-0186/

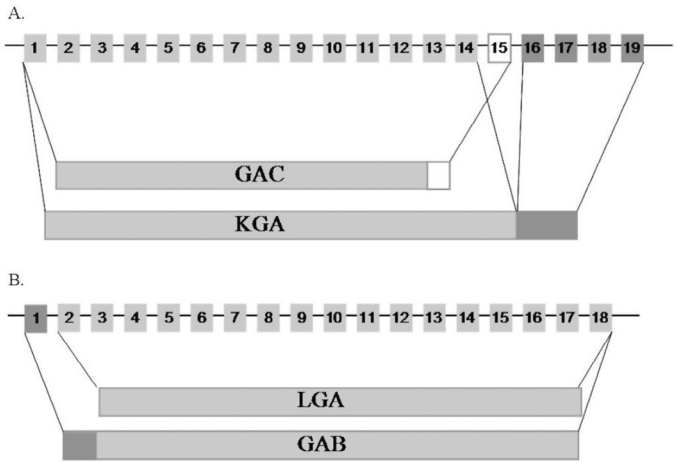

Fig. 1. Schematic diagram of *GLS* (A) and *GLS2* (B) and transcripts arising from these genes. (A) Two transcripts arise from *GLS*: human KGA mRNA is formed by joining exons 1–14 and 15–19, while human GAC mRNA arises from exons 1–15; therefore, human GAC protein differs from human KGA protein at the C-terminus. (B) Two transcripts arise from *GLS2*: human GAB mRNA contains exons 1–18, while human LGA mRNA lacks exon 1; therefore, human LGA protein differs from human GAB protein at the N-terminus. Exons are indicated as numbered boxes; introns are indicated as solid black lines. Different colors (white and dark gray) are used to distinguish exons involved in the generation of the C/N termini.

A schematic diagram of the human *GLS* and *GLS2* genes and transcripts arising from both genes is presented in Fig. 1.

Glutaminases have long been thought to be located exclusively in the mitochondria. However, in the central nervous system liver-type isoform shows also nuclear localization (Olalla et al., 2002). Moreover, the C terminus of GAB interacts with PDZ containing proteins (Olalla et al., 2001). Additionally, two ankyrin repeats were identified in the C terminus of GAB (Marquez et al., 2006). These observations suggest that besides the role as a classical enzyme, GAB behaves as a multifunctional protein (Marquez et al., 2006; Olalla et al., 2002).

4. Expression of glutaminase isoforms in brain tumors

Deregulated expression and/or activity of GA isoforms is a hallmark of neoplastic cell lines and tumors of different origin (Szeliga and Obara-Michlewska, 2009). Analysis of expression pattern of GA isoforms suggests that expression of kidney-type isoforms is correlated with high rate of cell proliferation (Pérez-Gómez et al., 2005; Turner and McGivan, 2003), whereas expression of liver-type isoforms is characteristic for resting or quiescent cells (Pérez-Gómez et al., 2005). Brain tumors present differential expression pattern of GA transcripts depending on their cellular origin. The lack or only traces of the isoforms encoded by the *GLS2* gene were found in tumors of astrocytic or ependymal origin, regardless of the grade of malignancy (Szeliga et al., 2005, 2008). The reasons for the diminished *GLS2* expression in gliomas are unclear. This gene has been shown to be a target of tumor suppressor p53 (Hu et al., 2010). Because p53 mutations are common in gliomas of astrocytic origin (Ohgaki and Kleihues, 2009), suppression of *GLS2* in these tumors could be a consequence of aberrant p53 function in these tumors. While the underlying mechanism remains to be studied, low GLS2 expression appears to be an exclusive property of tumor cell of glial origin. Of note in this context, considerable levels of *GLS2* transcripts were found in gangliogliomas containing both astrocytic and neuronal components (Szeliga et al., 2008). The expression of *GLS* gene has been shown to be induced by oncogene c-Myc and associated with cell proliferation (Gao et al., 2009). Isoforms encoded by the *GLS* gene are highly expressed in gliomas, regardless of the cellular composition and tumor grade (Szeliga et al., 2005, 2008). Of note, GAC is the most strongly expressed isoform in all brain tumors examined (Szeliga et al., 2008).

5. GAB suppresses malignant phenotype of glioblastoma

The presumption that GAB may play roles other than enzymatic led to the hypothesis that decreased level of this transcript in gliomas has implications for the physiology of these tumors. Transfection of T98G human glioblastoma cells with full-length GAB cDNA diminished cell proliferation, survival and migration (Szeliga et al., 2009). The exogenous GAB was catalically active, as transfected cells presented a decrease of Gln content and an increase of Glu content. Moreover, transfection with GAB altered the expression pattern of many genes of which some code for proteins relevant to malignancy (Szeliga et al., 2009). The molecular mechanisms underlying changes observed in the phenotype of transfected cells remain unknown. One of the hypotheses assumes that these alterations may be a consequence of changes in the cellular Gln level and in Gln/Glu ratio. There is a growing body of evidence that Gln affects expression of different genes, including transcription factors which in turn may alter expression of many other genes (Lu et al., 2009; Olalla et al., 2002; Wischmeyer, 2002). The other possibility is that GAB itself modifies expression of other genes. This concept relates to the hypothesis described above, assuming that GAB function goes beyond enzymatic activity. Studies aiming at unraveling the mechanisms underlying the puzzling GAB-dependent suppression of malignant phenotype of glioblastoma are under way in our laboratory.

One of the genes downregulated in the cells transfected with GAB is the *MGMT* gene encoding O^6-methylguanine-DNA methyltransferase, a DNA repair protein that removes alkyl groups from guanine in DNA to its own active center (Pegg et al., 1995). Alkyl groups are attached to DNA by the alkylating agents thereby resulting in apoptosis (Roos et al., 2007) or autophagy (Lefranc et al., 2007). Alkylating agents such as temozolomide (TMZ) and carmustine (BCNU) are used in glioma therapy. Decreased MGMT level results in the reduced ability to repair DNA and correlates with the clinical benefit of alkylating chemiotherapeutics (Weller et al., 2013). Downregulation of the *MGMT* gene in cells transfected with GAB resulted in a reduced amount of MGMT protein and its activity which in turn led to an increased sensitivity to both TMZ and BCNU (Szeliga et al., 2012). The molecular mechanism by which overexpression of *GLS2* is translated to downregulation of *MGMT* remains unknown. Promoter hypermethylation is one of the main ways that *MGMT* is epigenetically silenced (for review see Jacinto and Esteller, 2007). Transfection of T98G cells with GAB did not change the methylation pattern of *MGMT* promoter (Szeliga et al., 2012), suggesting that there are other mechanisms linking downregulation of *MGMT* with overexpression of GAB.

6. Opposite effects of *GLS* and *GLS2* on growth of glioblastoma cells

Research conducted in the past decade indicates that GA isoforms play opposing roles in tumorigenesis. It has been speculated that expression of K-type GA isoforms is correlated with high rate of cell proliferation (Pérez-Gómez et al., 2005; Turner and McGivan, 2003), whereas expression of L-type GA isoforms is associated with low proliferation rates and is characteristic for resting or quiescent cells (Pérez-Gómez et al., 2005). Knocking-down of *GLS* gene in the mouse mammary tumor cells (Lobo et al., 2000), the human breast cancer cells (Donadio et al., 2008), or glioblastoma cells (Cheng et al., 2011), led to a reversion of the transformed phenotype. As similar effects were attained by overexpression of the *GLS2* gene in glioblastoma